

**EVALUATION OF APICAL SEALING ABILITY AND
ANTIBACTERIAL EFFICACY AGAINST E.FAECALIS OF TWO
RESIN BASED SEALERS MIXED WITH PROPOLIS - AN IN VITRO
STUDY**

*A Dissertation submitted
in partial fulfillment of the requirements
for the degree of*

MASTER OF DENTAL SURGERY

BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS



THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI – 600 032

2008 – 2011

ACKNOWLEDGEMENT

I wish to place on record my deep sense of gratitude to my mentor **Dr. M. KAVITHA, MDS.**, for the keen interest, inspiration, immense help and expert guidance throughout the course of this study as professor & HOD i/c of the Dept. of Conservative Dentistry and Endodontics, Tamilnadu Govt. Dental College and Hospital, Chennai.

It is my immense pleasure to utilize this opportunity to show my heartfelt gratitude and sincere thanks to **Dr.S.JAIKAILASH,MDS.,D.N.B.**, Associate Professor & Guide of the Department of Conservative Dentistry and Endodontics, Tamilnadu Govt. Dental College and Hospital, Chennai for his guidance, suggestions, source of inspiration and for the betterment of this dissertation.

I take this opportunity to convey my everlasting thanks and sincere gratitude to **Dr. K.S.G.A. NASSER, MDS.**, Principal, Tamilnadu Government Dental College and Hospital, Chennai for permitting me to utilize the available facilities in this institution.

I am extremely grateful to **Dr.T. SABEETHA, M. D.(PATH) ,P.hd,asst. professor ,Madras Medical College, Chennai** for her guidance, suggestions and unconditional support to all my needs made this study feasible.

My sincere thanks to **Mrs. Vasanthi** for helping me throughout the process of my study regarding antimicrobial activity.

My sincere thanks to **Dr. BASKAR, M. Tech., P hd, Dept. of Biotechnology, Indian Institute of Technology, Chennai** for providing me opportunity to use stereomicroscope and special thanks to **Mr. Pundrik** who helped me in carrying out stereomicroscopic analysis.

I sincerely thank **Dr. B. Rama Prabha, MDS., Dr. K. Amudha Lakshmi, MDS., Dr. G. Vinodh, MDS., Dr. D. Aruna Raj, MDS., Dr.Nandhini. M.D.S., and Dr. Shakunthala. M.D.S.,** Assistant Professors for their suggestions, encouragement and guidance throughout this study.

I specially thank, **Dr.R. Ramanan** M.Sc, M.Phil., Ph.D.,Professor of Statistics, Presidency College, Biostatistician for all his statistical guidance and help.

I am always grateful to God Almighty, who is always beside me.

DECLARATION

TITLE OF DISSERTATION	EVALUATION OF APICAL SEALING ABILITY AND ANTIBACTERIAL EFFICACY AGAINST E.FAECALIS OF TWO RESIN BASED SEALERS MIXED WITH PROPOLIS - AN IN VITRO STUDY
PLACE OF THE STUDY	Tamil Nadu Government Dental College & Hospital, Chennai – 3.
DURATION OF THE COURSE	3 YEARS
NAME OF THE GUIDE	DR. S. JAIKAILASH
HEAD OF THE DEPARTMENT	DR. M. KAVITHA

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HOD i/c

GUIDE

Signature of the Candidate

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Introduction

INTRODUCTION

Complete filling of the root canal system is one of the most important aspects of successful endodontic treatment. The success of endodontics revolves around the efficient preparation and obturation of the apical third of root canal. By this procedure we expect the achievement of perfect seal at the apex and thus the elimination and future exclusion of all microorganisms from the root canal system²⁹.

Microorganisms infecting the root canal dentine might adhere superficially to the dentinal wall or penetrate deeper into the dentinal tubules⁶³. Superficially adhering bacteria might be expected to be killed easier than those shielded in the depths of dentinal tubules, but microorganisms inside the dentinal tubules might also be challenged by antimicrobial components leaching from the sealer.

The success of endodontic treatment depends mainly on elimination of infecting microorganisms. This is achieved through chemo-mechanical preparation of root canals and leaving antimicrobial dressings in the root canal between appointments. However, microorganisms might still survive these challenges^{58, 62}.

Gutta-percha is considered an impermeable core material; therefore, leakage through an obturated root canal is expected to take place at the interfaces between the sealer and dentin or the sealer and gutta percha, or through voids within the sealer. Hence, the sealing quality of a root canal filling also depends on the sealing ability of the sealer⁷⁶.

Enterococcus faecalis is a gram-positive bacterium often isolated in persistent root canal infections. Furthermore, it can penetrate deeply into dentinal tubules and resist bactericidal substances commonly used in endodontic procedures⁵³.

Therefore, antimicrobial testing of sealers should take into consideration these two effects based on the contact of the sealer and the microorganism. *Enterococcus faecalis* is a resilient bacterium frequently recovered from obturated root canals with signs of apical periodontitis . When established in the dentinal tubules, it is difficult to eliminate this species through root canal medication. Therefore, it might be advantageous if the sealer exerts some antimicrobial activity as the last element in the treatment regimen²⁸.

AH Plus is an epoxy resin-based cement derived from AH 26, which was introduced in 1954¹⁷.

MetaSEAL (Parkell Inc, Farmington, NY), a fourth generation methacrylate sealer has been recently introduced as another option for root canal obturation. This methacrylate resin-based sealer also has adhesion to the radicular dentin and to solid filling materials^{13, 74} .

Propolis is a natural, non-toxic resinous substance that has been collected from several types of plants by bees for covering and protecting the hive^{10, 19}. Currently, propolis has been employed in Medicine and Dentistry because of its anti-inflammatory, antiseptic, healing and antimicrobial properties³⁰.

Therefore, root canal sealers with good sealing ability and antimicrobial activity are desired to entomb and kill the surviving microorganisms.

Aim & Objectives

The aim of the present study is

- (1) To evaluate the apical sealing ability of two resin based sealers, AH plus and Metaseal, mixed with the antibacterial substance propolis using conventional gutta-percha obturation method and
- (2) To determine their antimicrobial activity against common endodontic pathogen *E. faecalis*.

Review of Literature

The continued research on obturation materials is based on the concept that the primary cause for failure of root canal treatment is the apical migration of microorganisms and their by products in a poorly filled and leaking root canal obturation.

Grossman (1982)³¹ studied the physical properties of filling materials and found adhesion to be a very desirable property in root canal cements.

Steinberg et al. (1986)⁷¹ investigated the antibacterial properties of Propolis and honey against oral bacteria in vitro and in vivo. Propolis demonstrated an in vitro antibacterial effect on both isolated oral streptococci and salivary bacterial counts in the clinical study.

Zidan O et al. (1987)⁷⁷ at the University of Minnesota, the efficacy of four different dentin bonding agents used as root canal sealers was tested. “No leakage was measurable in 75% of the canals sealed with Scotchbond (3-M) in 70% of canals sealed with Restodent (Lee Pharmaceuticals), in 60% of canals sealed with DentinAdhesit (Ivoclar), and in only 30% of canals sealed with GLUMA (Bayer Dental). The same researchers reported the “dramatic improvement in the quality of sealing root canals using dentin bonding agents.”The Minnesota study returned to single-cone gutta-percha filling with

the adhesives, the cone inserted undoubtedly spread the adhesive laterally and to occupy space to reduce shrinkage.

Caicedo et al. (1988)¹² reiterated that endodontic cements must seal the root canal space and, ideally, should adhere to both the gutta-percha cone and the canal walls.

Magro-Filho and de-Carvalho (1990)⁵¹ examined cutaneous wound healing and socket wound after tooth extraction in rats with topical application of either a 10% hydro alcohol solution of propolis or 10% hydro-alcohol solution alone. It was concluded that topical application of propolis hydro-alcoholic solution accelerated oral epithelial repair after root extraction but had no effect on socket wound healing.

Lonita et al (1990)⁴⁹ used a paste made from an alcoholic solution of propolis and zinc oxide. The study included 150 teeth with indirect pulp capping of deep cavities and 50 teeth with direct pulp capping. The results obtained showed that the paste with Propolis exerted effects similar to those of zinc eugenol. The morphologic study of the indirect capping showed that secondary dentin developed shortly after the application of the paste, and that it was followed by the development of pulpitis and sclerotic transformation of the pulp. In teeth

with direct capping a protective film developed at the opening of the pulp chamber.

Molloy D et al. (1992)⁶⁰ experimented with a Bis GMA unfilled resin as a sealer. The new material was found to be biocompatible but impossible to remove.

O. Zmener, C. Spielberg , F. Lamberghini & M. Rucci (1997)⁶¹ In this study an epoxy-resin-based endodontic sealer, AH Plus, was tested *in vitro* for apical leakage. The conventional sealer AH26 was used as the control. The root canals of 72 single-rooted teeth were prepared biomechanically using a stepback technique before lateral condensation of gutta-percha with one of the two sealers. Teeth were immersed in 5% methylene blue dye for 2, 4 or 10 days. The roots were split longitudinally so that the extent of dye penetration could be measured with a stereomicroscope. The mean extent at 2 days was 0.4 mm for AH26 and 1.4 mm for AH Plus. Neither material produced a complete apical seal and leakage increased with the duration of immersion in dye.

Jose F. Siqueira et al. (2000)⁴³ investigated and compared the antimicrobial effects and the flow rate of the following sealers: Kerr Pulp Canal Sealer EWT, Grossman's Sealer, ThermaSeal, Sealer 26, AH Plus, and Sealer Plus. The agar diffusion test was used to assess the antimicrobial activity of the sealers. All

root canal sealers tested showed some antimicrobial activity against most of the microorganisms. There were no significant differences between the materials tested. All root canal sealers also flowed under the conditions of this study. Statistical analysis of the results revealed that AH Plus and Kerr Pulp Canal Sealer EWT had flow values significantly superior to the other sealers tested.

Mario Roberto Leonardo et al. (2000)⁵² evaluated the antimicrobial activity of four root canal sealers (AH Plus, Sealapex, Ketac Endo, and Fill Canal), two calcium hydroxide pastes (Calen and Calasept), and a zinc oxide paste. Seven bacterial strains were used, six of them standard. Activity was evaluated using the agar diffusion method with Brain Heart Infusion agar and Muller Hinton medium seeded by pour plate. Calcium hydroxide based sealers and pastes were either placed directly into 4.0 x 4.0 mm wells or by using absorbent paper points. The plates were kept at room temperature for 2 hr for diffusion. After incubation at 37°C for 24 hr, the medium was optimized with 0.05 g% ITC gel and inhibition haloes were measured. All bacterial strains were inhibited by all materials using the well method. However, when the materials were applied with absorbent paper points, *Enterococcus faecalis* was not inhibited by zinc oxide, and *Pseudomonas aeruginosa* was not inhibited by AH Plus, Fill Canal, and the zinc oxide based paste.

I. Miletie, G. Prpie-Mehifie, T. Maruan, A. Tambie-Andrauevie, S. Pleuko, Z. Karlovie & I. Anie(2002)³⁸ evaluated the penetration of *Candida albicans* alone and a combination of bacteria through root canals filled with gutta-percha and one or other root canal sealers, AH26 and AH Plus. In this study, eighty teeth were randomly divided into two groups of 40 teeth each and obturated with gutta-percha using either AH26 or AH Plus sealer. Results showed leakage in the experimental teeth occurred between 14 and 87 days. Leakage was present in 47% of all samples. From the samples with AH26, 45% leaked bacteria and 60% leaked fungi; whilst from the samples with AH Plus, 50% leaked bacteria and 55% fungi. There was no statistically significant difference in penetration of bacteria and fungi between the sealers. They concluded that gutta-percha and the sealers AH26 and AH Plus allowed leakage of bacteria and fungi.

Kont C (2002)⁴⁶, et al evaluated the microleakage of root fillings involving four root-canal sealers including AH Plus, RoekoSeal, Ketac-Endo and Sultan. A fluid filtration method was used for quantitative evaluation of apical leakage. Results indicated that all the root fillings showed less leakage after 21 days. Fillings incorporating Sultan showed significantly more leakage than all other sealers. He concluded that root fillings with RoekoSeal in combination with cold lateral condensation technique showed better sealing than those with Ketac-Endo, AH Plus and Sultan sealers after 21 days.

Hind Al-Qathami, and Ebtissam Al-Madi(2003)³⁷ compared the antimicrobial activity of propolis with that of sodium hypochlorite in a root canal system. Forty-nine extracted human teeth with large carious lesions reaching the pulp were instrumented using step-back technique. Propolis, sodium hypochlorite and saline were used as irrigants. Microbiological samples were taken from the teeth immediately after accessing the canal, and after instrumentation and irrigation. The results of this study indicated that the propolis has antimicrobial activity equal to that of sodium hypochlorite.

G. Kayaoglu, H. Erten, T. Alac, & D. Ørstavik (2005)²⁸ studied the antimicrobial activity of root canal sealers on *Enterococcus faecalis*, either allowing or avoiding direct contact between sealers and bacteria. Methodology Filter paper discs were immersed in standardized *E. faecalis* suspensions and exposed to freshly mixed sealers (MCS, AH Plus, Grossman's sealer, Sealapex, Apexit) in teflon wells for 30 min, with or without a filter membrane placed between filter paper discs and sealers (membrane-restricted contact test and direct contact test, respectively).

Results showed that in the direct contact test, MCS and AH Plus killed the bacteria to a level below the detection limit. They were followed in decreasing order of efficacy by Grossman's sealer, Sealapex and Apexit. In the membrane-restricted contact test, the sealers ranked: MCS, AH Plus, Grossman's sealer,

Apexit and Sealapex, in descending order of antibacterial potency. Calcium hydroxide-based sealers, Sealapex and Apexit were ineffective in this short-term experiment.

FÁBIO DULTRA et al. (2006)²⁴ compared the apical sealing ability of four root canal sealers. forty extracted human maxillary canines were instrumented 1 mm short of the anatomical apex and randomly assigned to four groups (n=10), according to the root canal sealer used for obturation: Endofill, AH Plus, EndoREZ and Epiphany. Root canals were obturated with gutta-percha points, except for the Epiphany group, in which resin points (Resilon) were used. The teeth were immersed in India ink for seven days and clarified using methyl salicylate. The extent of apical dye penetration was measured with a measuroscope in all aspects of the canal. They concluded that the resin based root canal sealers presented lesser apical microleakage than the zinc oxide and eugenol based sealer. No statistical differences were observed among resin based sealers.

Funda Kont Cobankara Hasan Orucoglu, Abdülkadir Sengun, and Sema Belli,(2006)²⁷ evaluated the apical seal obtained with four root canal sealers: Rocanal 2, Sealapex, AH Plus, and RC Sealer. Forty root canals were prepared using the step-back technique. The specimens were divided into four groups of 10 samples and obturated by laterally condensed gutta-percha with one of the

tested sealers. The computerized fluid filtration method was used for evaluation of apical sealing properties. The quantitative apical leakage of each specimen was measured after 7, 14, and 21 days. Statistical analysis indicated that the apical leakage of all sealers used in this study decreased gradually from 7 days to 21 days. Sealapex showed better apical sealing than the other sealers at 7, 14, and 21 days. RC Sealer, AH Plus, and Rocanal 2 showed similar apical leakage values at every period.

Aravind, V Gopikrishna, D Kandaswamy, Rajan K Jeyavel (2006)⁵

undertook the study to evaluate the antimicrobial efficacy of a traditional zinc oxide eugenol based sealer (Tubliseal) with a iodoform incorporated zinc oxide eugenol based sealer (Endotas FS), a calcium hydroxide based sealer (Apexit) and the epoxy resin based sealers (AH PLUS and PC Seal), against the micro organisms *Enterococcus faecalis* and *Candida albicans*.

The antimicrobial efficacy of an iodoform incorporated zinc oxide eugenol based sealer, Endoflas FS against *Enterococcus faecalis* and *Candida albicans* was statistically superior to the rest of the test groups. Endoflas FS performed far better than even the controls being employed (Amoxycillin and Nystatin) respectively. Tubliseal, a zinc oxide eugenol based sealer also showed significant antimicrobial properties, but was statistically inferior to Endoflas FS. Apexit, a calcium hydroxide based sealer did not show significant antimicrobial efficacy against both *Enterococcus faecalis* and *Candida albicans*. AH PLUS

and RC seal, epoxy resin based sealers showed no antimicrobial properties whatsoever.

Serge Bouillaguet, John C. Wataha, Franklin R. Tay, Martha G. Brackett, and Petra E. Lockwood(2006)⁶⁹ measured the cytotoxicity of three endodontic sealers (AH Plus/Maillefer- Dentsply, Epiphany/Pentron, GuttaFlow, Coltene- Whaledent). Materials were mixed according to the manufacturer instructions and packed into Teflon molds (10 × 1 mm). For cytotoxicity testing (MTT method), the specimens were placed in contact with cultured cells, then evaluated at two subsequent time points (24 or 72 h). The results showed that most materials pose significant cytotoxic risks and that cytotoxicity generally increased with time. At 72 h, GuttaFlow became significantly less toxic than AH Plus, Epiphany sealer, and Resilon.

Luigi Pinna et al.(2008)⁵⁰, compared the cytotoxicity of MetaSEAL with an epoxy resin-based (AH Plus Jet) and a zinc oxide–eugenol-based sealer (Pulp Canal Sealer).

Five-millimeter diameter disks prepared from the respective sealer and disks prepared from Teflon and polymethyl methacrylate were placed in direct contact with a rat osteosarcoma (ROS) 17/2.8 rat osteoblast-like cell line at six intervals after setting completely at 72 hours and for 5 succeeding weeks after the disks were immersed in simulated body fluid. All sealers exhibited severe

toxicity at 72 hours, after which toxicity decreased gradually over the experimental period except for Pulp Canal Sealer, which remained severely toxic. MetaSEAL was more toxic than AH Plus Jet during the first week

Matthew et. Al (2008)⁵³ evaluated the dislocation resistance of root fillings created with MetaSeal. Forty-six incisors were cleaned and shaped using NaOCl and EDTA as irrigants. They were filled with gutta-percha/MetaSeal or gutta-percha/AH plus sealer using either a single-cone technique or warm vertical compaction (n=10). The roots were sectioned at the coronal and middle thirds to obtain thin slices, which were subjected to compressive loading to displace the set sealer/filling toward the coronal side of the slice. The remaining six teeth were filled with gutta-percha/MetaSeal and cryofractured for scanning electron microscopic examination. The push-out strength of AH plus was significantly higher than MetaSeal irrespective of filling techniques.

Saulius Drukteinis, Vytaute Peciuliene, Rasmute Maneliene, Ruta Bendinskaite (2009)⁶⁸ determined and compared the microbial leakage of roots filled with EndoREZ sealer/EndoREZ® Points and AH Plus sealer/conventional gutta-percha points.

For this, 60 single-rooted teeth were prepared using step-back technique. The smear layer was removed with 18% EDTA. In AH Plus group root canals were

obtured with AH Plus sealer/gutta-percha and in EndoREZ group with EndoREZ sealer/EndoREZ® Points. The coronal chambers were filled with the mix of human saliva and broth (ratio 3:1). The medium was changed every 7 days. Microbial growth in the broth was evaluated every day up to the end of experiment.

Results showed leakage in the root canals of the teeth from experimental groups between 4 and 75 days. The mean leakage in AH Plus group was 18.86 days, while in EndoREZ group it was 28.28. they concluded that both types of root fillings – EndoREZ sealer/EndoREZ® Points and AH Plus sealer/gutta-percha points – showed microbial leakage.

Brian R. Babb et al.(2009)⁹ examined the adhesive strengths, interfacial ultrastructure, and tracer penetration of a nonetching (EndoREZ) and two self-adhesive methacrylate resin–based sealers (MetaSEAL and RealSeal SE) when they were applied to radicular dentin following the manufacturers’ recommended use of EDTA as the active final rinse. A modified push-out testing design was used to evaluate the dislodgement of core-free sealers. The mixed sealers were placed in dimensionally identical, artificially created canal spaces prepared in the coronal, middle, and apical thirds of radicular dentin. After setting, each sealer-filled cavity was subjected to compressive loading until failure. Additional specimens were prepared for transmission electron microscopy to examine the ultrastructure and nanoleakage within the sealer-

radicular dentin interface. Results showed that the two self-adhesive sealers MetaSEAL and RealSeal SE exhibited higher push-out strengths than the nonetching sealer EndoREZ when EDTA was used as the active final rinse.

Claudia Ramos Pinheiro et al. (2009)¹⁴ studied to evaluate the sealing ability of AH Plus, Epiphany, Acroseal, Endofill, and Polifil after active lateral condensation technique, by using a bacterial test, during 64 days.

One hundred bovine incisors were cleaned and shaped; then they were filled with the endodontic sealers and adapted into a microcentrifuge tube. The setup root/microcentrifuge tube was added to glass flasks containing Brain Heart Infusion broth. A culture of *Enterococcus faecalis* was inserted into the upper chamber of each assembly. Daily leakage was evaluated through the broth turbidity.

Conclusions were: AH Plus and Endofill had the worst sealing ability when compared with Polifil, which showed the least leakage. Acroseal and Epiphany showed a tendency toward having an intermediate behavior; however, there was no significant difference among Acroseal, Epiphany, and the other sealers.

Emel Olga Onay, Mete Ungor, Saadet Unver, Hale Ari, and Sema Belli (2009)²¹ compared the short-term sealing abilities of recently introduced polymeric endodontic filling systems.

Root canals of 120 extracted and decoronated human single rooted teeth were instrumented using crown-down technique with HERO Shaper rotary instruments. The roots were divided randomly into 8 groups and filled with different combinations of core and sealer as follows: group 1, RealSeal/Resilon; group 2, RealSeal/Herofill; group 3, Hybrid Root Seal/Resilon; group 4, Hybrid Root Seal/Herofill; group 5, MM-Seal/Resilon; group 6, MM-Seal/Herofill; group 7, positive controls (Herofill only); group 8, negative controls. Apical leakage quantity was evaluated after 1 week by using a fluid filtration model. For each sample, measurements of fluid movement were recorded at 2-minute intervals for a total of 8 minutes, and then averaged.

Results showed that, of all the groups, MM-Seal/Herofill combination exhibited the least microleakage, and RealSeal/Herofill combination ranked second in this regard. The mean leakage values for the RealSeal/Resilon and MM-Seal/Resilon combinations were both significantly higher than the means for the other 4 experimental groups. Hybrid Root Seal combined with Resilon resulted in significantly less microleakage than Hybrid Root Seal combined with Herofill.

Sui Mai, DDS, Young Kyung Kim, Noriko Hiraishi, Junqi Ling, David H. Pashley, and Franklin R. Tay(2009)⁷² evaluated the true self-etching potential of MetaSEAL.

Mixed MetaSEAL sealer was applied to (1) fractured radicular dentin that was devoid of smear layers, (2) instrumented canal wall radicular dentin that was irrigated with water as the final rinse to preserve the smear layer, and (3) instrumented canal wall radicular dentin that was irrigated with ethylenediaminetetraacetic acid (EDTA) as the final rinse to remove the smear layer. Cryofractured tooth halves without sealer application were examined by scanning electron microscopy to identify the characteristics of the bonding substrates.

They found that MetaSEAL did not demineralise fractured radicular dentin that was devoid of smear layer and smear plugs. The self-adhesive sealer was incapable of etching beyond the 1- to 2-mm-thick smear layer retained on water-irrigated instrumented dentin to demineralize the underlying radicular dentin. They concluded that the limited self-etching potential of MetaSEAL is a clinically legitimate concern.

Lama Awawdeh, Maha AL-Beitawi, and Mohammad Hammad(2009)⁴⁷ investigated the antimicrobial activity of propolis-based intracanal medicament against *Enterococcus faecalis* using infected dentine models, and compared its

antimicrobial efficacy with that of the non-setting calcium hydroxide paste when used as a short-term medication for 1 and 2 days. Results showed that propolis was significantly more effective than non-setting calcium hydroxide against *E. faecalis* after short-term application. They concluded that propolis is very effective as intracanal medicament in rapidly eliminating *E. faecalis* *ex vivo*.

Richard Stoll et al.(2010)⁶⁶, evaluated the bond strength of different adhesive sealers on Resilon and gutta-percha. Pellets of gutta-percha and Resilon were embedded into test tubes. Small eyelets were attached to those surfaces with a 0.5-mm film of different root canal sealers. Real Seal, Real Seal SE, Hybrid Root Seal (Meta Seal), and AH Plus were used. AH Plus as a nonadhesive sealer served as a control group. In all groups (n = 10) shear bond strength was measured.

Results: Shear bond strength was relatively low (0.1–3 MPa) and significantly higher in the groups with a single component adhesive sealer. No substantial bond strength was found in the control group. Overall bond strength to Resilon was higher than to gutta-percha but not significant compared with the Hybrid Root Seal group. Conclusion was made that with single component self-adhesive sealers, an adhesive connection might be formed to gutta-percha as well as to Resilon.

A. S. Al-Hiyasat, M. Tayyar & H. Darmani(2010)² investigated the cytotoxic effects of four resin-based root canal sealers, namely, AHPlus, EndoREZ, Epiphany, and Metaseal, one of the latest generation methacrylate 4-META–containing resin-based sealers. The materials were mixed according to the manufacturer’s instructions, and elutes of the materials were prepared by incubating 1 g of the endodontic sealers in sterile phosphate-buffered saline for 1 week at 37 _C. The elutes were incubated with Balb C 3T3 fibroblasts for 48 h, and cytotoxic activity was measured using the MTT assay, which tests for mitochondrial enzyme activity.

Results showed that all materials were cytotoxic to different degrees. AHPlus was the least cytotoxic followed by EndoRez, Epiphany and Metaseal, which was the most cytotoxic.

Hale Ari, Sema Belli and Betul Gunes(2010)³⁵ performed a study to evaluate the apical sealing ability of Hybrid Root SEAL (MetaSEAL) in conjunction with different obturation techniques.

Sixty-eight extracted human mandibular straight single-rooted teeth with mature apices were prepared using a step-back technique and divided into 4 experimental groups (n _ 15). The experimental groups were obturated with Hybrid Root SEAL (MetaSEAL) using cold lateral condensation, vertical condensation, Thermafil, and Ultrafil techniques. Fluid movement along the

filled canals was measured using a fluid filtration method. Measurements were made at 2-minute intervals for 8 minutes.

Results showed that cold lateral and vertical condensation had significantly less fluid movement than the Thermafil and Ultrafil groups. Thermafil group had the highest fluid movement values when compared with the other groups.

It was concluded that Hybrid Root SEAL (MetaSEAL) had less fluid movement with cold lateral and vertical condensation techniques when compared with Thermafil and Ultrafil techniques.

Emel Olga Onay, Hasan Orucoglu, Arlin Kiremitci, Yonca Korkmaz, and Gizem Berk(2010)²⁰ studied the sealing ability of 2 different resin-based endodontic filling systems after smear layer removal with 2 different techniques.

Extracted human single-rooted teeth (n =74) were instrumented using HERO Shaper rotary instruments and irrigated with 1 mL of 2.5% NaOCl between each instrument. Additionally, the canals received either an extra 3-minute rinse with 2 mL of 17% EDTA or a 40-second Er,Cr:YSGG laser treatment. The root canals were filled with either Hybrid Root Seal/Resilon combination or AH Plus/gutta-percha combination using lateral condensation technique (n = 11). Apical leakage quantity was measured with the computerized fluid filtration meter at 1 and 4 weeks. One root from each group, which was not submitted to

the fluid filtration test, was selected for scanning electron microscopy (SEM) analysis.

Results: A significant decrease was observed in the microleakage values of all the experimental groups tested with time. EDTA _ AH Plus/gutta-percha combination exhibited the least microleakage, whereas laser irradiation _ Hybrid Root Seal/Resilon combination showed the greatest microleakage at each of the 2 time periods. Each experimental combination exhibited architecture in SEM that seemed to correlate with its sealing performance.

Conclusion was made that Er,Cr:YSGG laser treatment does not enhance the sealing ability of the sealers compared with EDTA application. The root canal adaptation and sealing ability of the Hybrid Root Seal/Resilon combination is not superior to that of the AH Plus/gutta-percha combination.

Meriç Karapınar-Kazandag et al. (2010)⁵⁴ evaluated the microleakage of Resilon _ Epiphany, EndoRez, Activ GP, and conventional AH Plus _ gutta-percha technique using the glucose filtration model.

One hundred twenty maxillary incisors were divided into 5 experimental and 2 control groups. After root canal shaping, the experimental groups were filled with AH Plus _ gutta percha (lateral compaction), Resilon _ Epiphany (lateral compaction), AH Plus _ Protaper's proprietary cone, EndoRez _ sealer, or Activ GP _ sealer). The specimens were mounted on a glucose model and samples were taken for 3 weeks for leakage measurement.

Result showed no statistically significant difference. They concluded that the filling materials with the monoblock concept do not seem to be superior to the conventional AH Plus _ gutta-percha system regarding microleakage.

J. Santos et al.(2010)⁴⁰ evaluated the ability of two resin-based filling materials to provide immediate and long-term sealing of the root canal. For this, a total of eighty-two human roots were instrumented and filled with AH Plus/gutta-percha or Epiphany/Resilon. The quality of root canal sealing was assessed by a fluid filtration method performed at immediate and 180-day time intervals. Results Specimens filled with Epiphany/Resilon exhibited higher leakage than specimens filled with AH Plus/gutta-percha, regardless of the coronal sealing condition and period of evaluation. Conclusions made were, AH Plus/gutta-percha provided superior root canal sealing at both immediate and 180-day time periods, the presence of a coronal seal reduced leakage significantly and storage of root filled specimens did not disturb the sealing ability of the tested materials.

Materials and Methods

ARMAMENTARIUM:

FOR APICAL SEALING ABILITY:

For decoronation of teeth

- 50 single rooted anterior teeth
- Diamond coated disc with mandrel
- Slow speed straight handpiece

For root canal treatment

- K- files No. 15 – 40 (Dentsply)
- K- files No. 45 – 80 (Dentsply)
- Endogauge (Dentsply)
- Slow speed micromotor handpiece
- 5% sodium hypochlorite
- Chelating agent (ethelyne diamine tetraacetic acid)
- Normal saline
- Tweezers
- 2 ml syringe
- Burnisher

- Spirit lamp
- Light curing unit
- Paper mixing pads
- Spreaders (No. 15 -40) (Dentsply)
- IRM (Dentsply)
- Gutta-percha points (15-60 sizes)(0.02 taper) (Dentsply)
- Paper points (0.02 taper) (Dentsply)

For specimen preparation

- Slow speed micromotor straight handpiece
- Diamond coated toothed disc
- Incubator
- Nail varnish
- Yellow sticky wax
- Rhodamine B dye (pH – 7) (Chenchems)

For testing of specimens

- Optical Stereomicroscope (Nikon, Japan)

Materials used:

➤ **AH PLUS SEALER (DENTSPLY)**

It is an epoxy resin based sealer supplied as paste- paste system.

Composition:

AH Plus Paste A

(Epoxy Paste)

- Bisphenol-A epoxy resin
- Bisphenol-F epoxy resin
- Calcium tungstate
- Zirconium oxide
- Silica
- Iron oxide pigments

AH Plus Paste B

(Amine Paste)

- Dibenzoldiamine
- Amino adamantane
- Tricyclodecane-diamine
- Calcium tungstate
- Zirconium oxide
- Silica
- Silicone oil

➤ **MetaSEAL SEALER (PARKELL INC.,FARMINGDALE, NY)**

It is a 4-META based, self-etch, dual cure resin sealer

Composition:

Powder

- Zirconium oxide filler,
- SiO₂ filler, and
- Polymerization initiators.

Liquid

- 4- META(4 methacryloyloxyethyl trimellitate anhydride)
- HEMA (2-hydroxy ethyl methacrylate)
- Dimethacrylate

➤ **PROPOLIS PLATINUM (K-LINK PHARMA)**

METHODOLOGY

50 freshly extracted single rooted human anterior teeth were used for the study.

INCLUSION CRITERIA:

Single and straight canal

Caries free teeth

Teeth with completely formed apices

SAMPLE PROCESSING

The teeth were rinsed under tap water in order to remove blood & tissue debris.

Soft tissue tags, bone or calculus was removed and then teeth were stored in normal saline at room temperature until use.

PREPARATION OF THE SAMPLES

All teeth were decoronated using diamond coated toothed disc, under continuous water spray leaving 13mm of root length for standardization.

Patency of the canal was established using No. 10 K file. The working length was established at 1mm short of the length of the file at the point where it just exited the root.

Instrumentation was performed using K-files (Dentsply) with a step back technique. 5% NaOCl was used as intermittent irrigant after each file. Canals are enlarged till ISO standard #60. On completion of instrumentation, smear layer was removed by rinsing the canal with EDTA, pH: 7.3 for over 3 to 5 mins. Canal was ultimately rinsed with normal saline to remove all chemicals.

DISTRIBUTION OF SAMPLES

Specimens (**n=50**) were divided into 5 groups:

Group I (n=10): control group (canals are obturated only with gutta-perch without sealer)

Group II (n=10): AH plus sealer

Group III (n=10): MetaSEAL sealer

Group IV (n=10): AH plus sealer mixed with propolis

Group V (n=10): MetaSEAL sealer mixed with propolis

Following distribution of sample, master gutta-percha point (0.02 taper, ISO No. 60) was trial fitted and trimmed if required to achieve tug back. Each canal was then dried with 0.02 taper paper points (Dentsply) and subsequently obturated depending on the group it belonged to, using lateral condensation technique.

For respective groups sealers are mixed according to manufacturer's instructions. After drying the canal with paper points, mixed sealer was coated onto the root canals with the help of the master gutta-percha cone. The prefit master cone (0.02 taper resin coated, ISO N0. 60) was then inserted into the canal to the working length, followed by the placement of multiple, accessory 0.02 taper, gutta-percha cones until the canal is completely filled and accessory cones are no longer going more than 2 mm in the canal.

Group 1 (n=10): control group

In this group, canals are obturated using only gutta-percha without use of any sealer.

Group 2 (n = 10): AH plus sealer

Sealer was mixed according to manufacturer's instructions. An equal length of AH plus paste A and paste B were dispensed on the mixing pad and mixed until uniform colour achieved.

Later, canals were obturated as described above.

Group 3 (n = 10) : MetaSEAL sealer

Sealer was mixed according to manufacturer's instructions. One scoop of powder was dispensed with 3 drops of liquid and mixed until a paste consistency was achieved.

After obturation using lateral condensation technique, the coronal portion of the sealer was light cured for 40 sec, to stabilize the material, enabling excess gutta-percha to be removed with a hot instrument.

Group 4 (n = 10) : AH plus mixed with propolis

Sealer was mixed according to manufacturer's recommendations and then into that mixture 4% of propolis was added with sterile micropipette. These were mixed until uniform colour is obtained.

Canals were obturated as described above.

Group 5 (n = 10) : MetaSEAL mixed with propolis

After mixing of the sealer 4% of propolis was added to the mix using micropipette and mixed till uniform colour obtained.

Each canal was obturated using this mix with lateral condensation technique described above and light cured for 40 seconds.

All the teeth thus obturated, in all the groups were stored in 100% humidifier at 37°C in the incubator for 48hrs to ensure complete setting of the sealer.

Coronal 2mm of the filling was removed with heated instrument for all the specimens to allow to seal the coronal end with IRM to prevent coronal leakage during dye penetration.

Each root was then coated with 2 coats of nail varnish, upto 2mm of apex including the coronal surface. Each layer was allowed to dry completely before second application. After varnish application, teeth were coated with yellow sticky wax exposing only apical 2 mm of teeth.

Specimens were then placed horizontally in 1% Rhodamine dye for 24 hours for passive dye penetration. After 1 day, they were washed in running tap water for 5 minutes and yellow sticky wax and nail varnish scraped off with a scalpel. Specimens were then divided for observation under stereomicroscope.

STEREOMICROSCOPIC EXAMINATION

Specimens from each group were divided into 2 halves buccolingual vertical sections.

Each root piece was serially sectioned vertically with a slow speed, diamond coated toothed disc under water cooling.

Each wider buccolingual section was examined under stereomicroscope at 2x magnification from apical end and continued coronally till the leakage could be observed.

Extent of leakage was measured in millimetre from apical to coronal end.

FIG. 1 50 SINGLE ROOTED HUMAN ANTERIOR TEETH

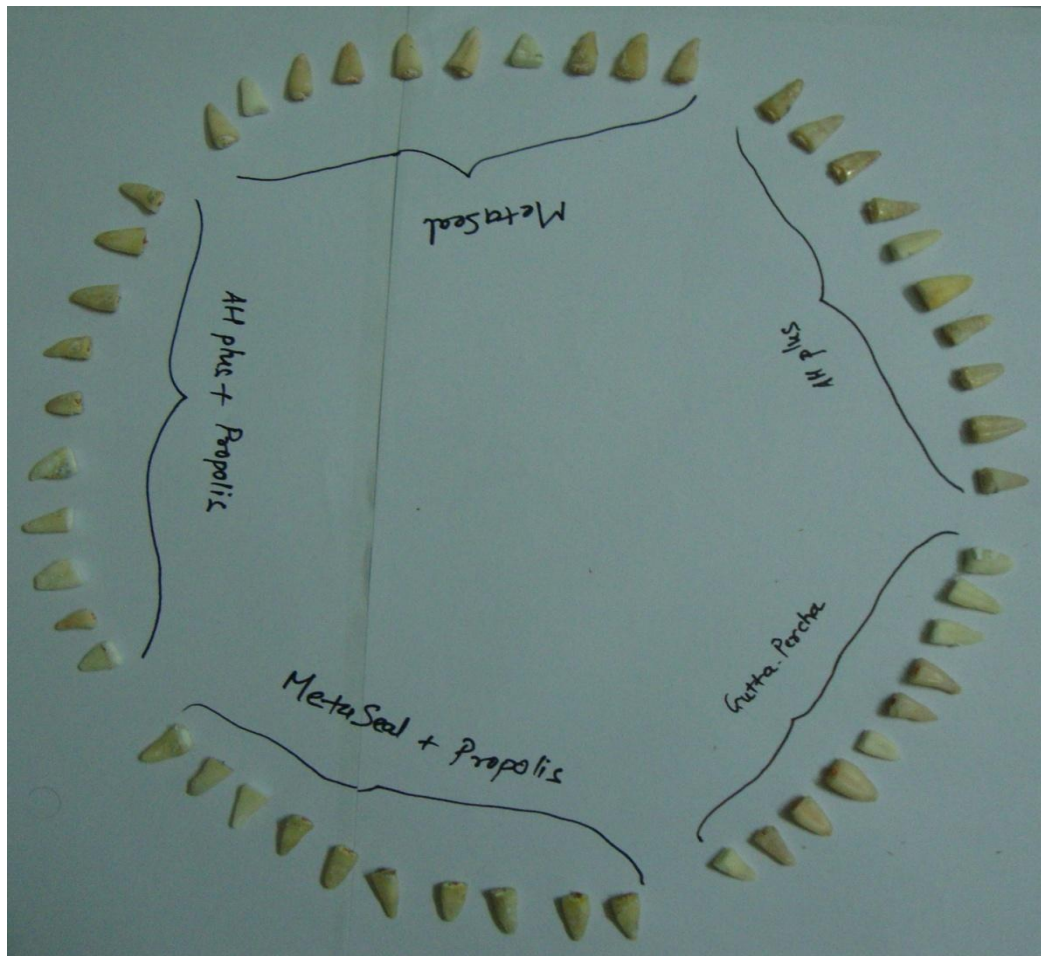


FIG. 2 MICROMOTOR HANDPIECE AND DIAMOND DISC WITH MANDREL



FIG. 3 ARMAMENTARIUM FOR ROOT CANAL TREATMENT



FIG. 4 IRRIGANTS USED



FIG. 5 BIOMECHANICAL PREPARATION WITH K-FILES



FIG . 6 ROOT CANAL SEALERS



FIG. 7 SEALERS WITH MIXING PADS AND SPATULAS



FIG. 8 PROPOLIS



FIG. 9 MICROPIPETETTE USED TO MEASURE PROPOLIS



FIG. 10 AH PLUS SEALER AFTER MIXING



FIG. 11 MetaSEAL SEALER AFTER MIXING



FIG. 12 DISPENSING SEALER WITH PROPOLIS



FIG. 13 AH PLUS MIXED WITH PROPOLIS



FIG. 14 MetaSEAL MIXED WITH PROPOLIS



FIG. 15 APPLICATION OF SEALER WITH MASTER GUTTA-PERCHA POINT

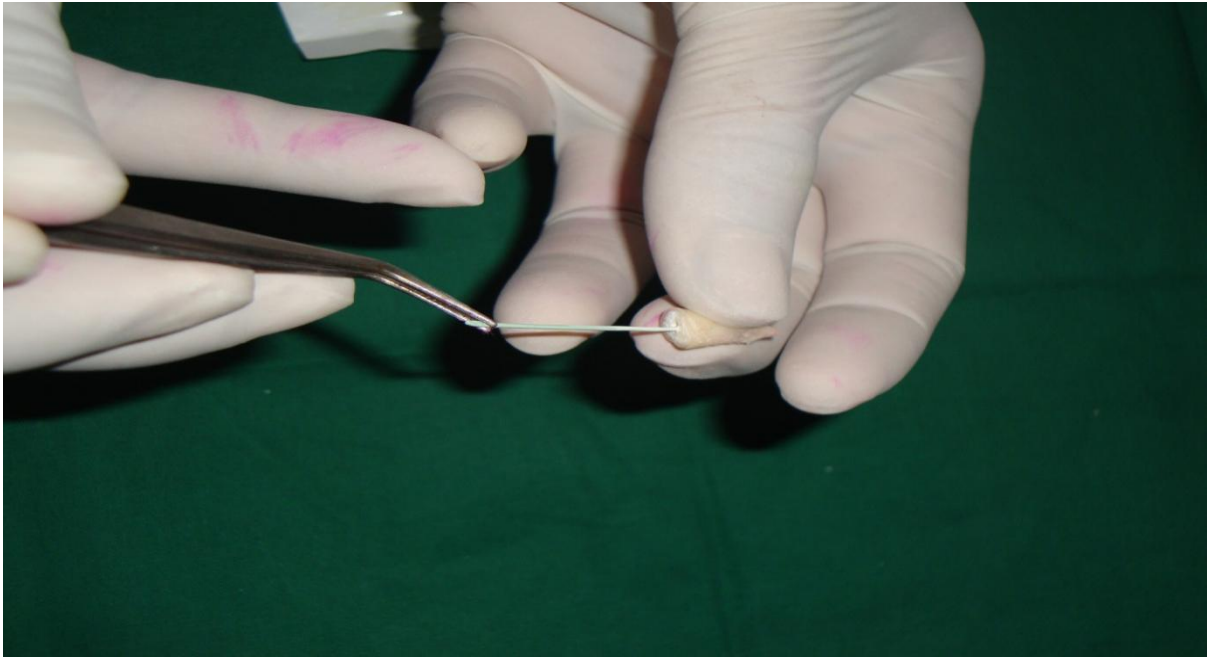


FIG. 16 LIGHT CURING TO PROVIDE IMMEDIATE CORONAL SEAL FOR MetaSEAL



FIG. 17 NAIL VARNISH, YELLOW STICKY WAX AND RHODAMINE DYE



FIG. 18 SPECIMES COATED WITH NAIL VARNISH



FIG. 19 SPECIMENS COATED WITH YELLOW STICKY WAX



FIG. 20 SPECIMENS IMMERSED IN RHODAMINE DYE



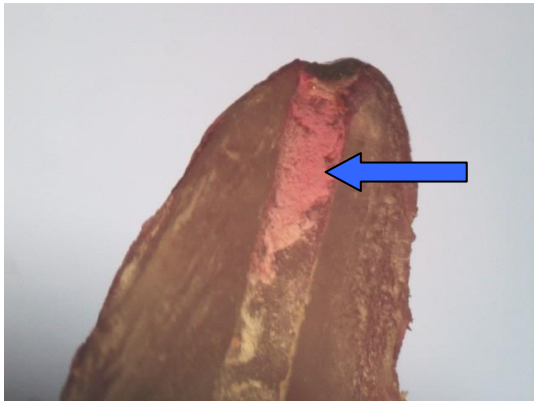
FIG. 21 SPECIMENS SECTIONED LONGITUDINALLY



FIG. 22 OPTICAL STEREOMICROSCOPE



FIG. 23 STEREOMICROSCOPIC VIEW OF REPRESENTATIVE SAMPLES



AH PLUS



MetaSEAL

**DYE
PENETRAT
ION**



CONTROL GROUP (ONLY GUTTA-PERCHA)



**AH PLUS MIXED WITH
PROPOLIS**



**MetaSEAL MIXED WITH
PROPOLIS**

FOR ANTIBACTERIAL ACTIVITY:

Armamentarium :

- 6 petri dishes
- Micropipette
- Incubator
- Autoclave
- Mixing pads for sealers
- AH plus and MetaSEAL sealers
- Mixing spatulas
- Mueller-Hinton agar
- Propolis
- Trypticase Soy Broth (TSB)

METHODOLOGY :

Agar diffusion test was used to measure the antibacterial activity.

Preparation of culture media:

For the agar diffusion test (double layer agarwell technique), a base layer composed of 10.0 mL of Mueller hinton agar with addition of 2% bacteriological agar was poured into sterile Petri dishes.

E. faecalis ATCC 29212 was reactivated from lyophilized stock frozen stock for observation of cell and colony morphology(Madras Medical College, Chennai). The microorganism was reactivated after incubation at 37°C for 24 h. For the inoculum, the culture in broth incubated at 37°C for a period of 15 to 18 h was used to standardize the final concentration of 1.5×10^8 cells/mL equivalent to the 0.5 standard of the McFarland scale.

Immediately after removal from the incubator, the bacterial inocula were seeded with cotton sticks all over the dishes, based on the McFarland scale, using Trypticase Soy Broth (TSB).

After solidification of the seed layer, five 6 x 4 mm wells were made in each dish by removal of the agar at equidistant points using a sterile straw, and were immediately filled with the test and control materials (one well for each substance). Six repetitions of the test were done, that is, 6 plates were used for the test microorganism.

Mixing of sealers and placement:

Following groups were used for evaluation:

Group A (n=6): control group (normal saline)

Group B (n=6): AH plus sealer

Group C (n=6): MetaSEAL sealer

Group D (n=6): AH plus sealer mixed with propolis

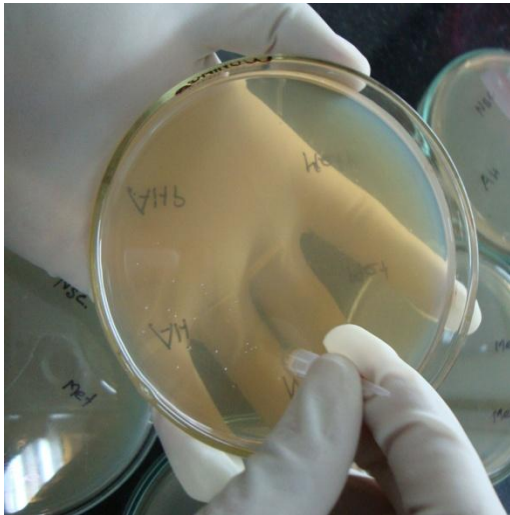
Group E (n=6): MetaSEAL sealer mixed with propolis

All the sealers in group I and II were mixed according to manufacturer's recommendations.

In group III and IV, Propolis was mixed in 4% using micropipette with AH plus and MetaSEAL sealers respectively.

After placement of the materials, each dish was kept at room temperature for 2 h for pre-diffusion of the material and then incubated at 37°C for 24 up to 48 h. After the initial 24 h, the zones of microbial growth inhibition around the wells were measured with a millimeter ruler with 0.5-mm accuracy. The dishes were incubated again and new measurements were done after 48 h.

**FIG. 24 PREPARATION OF
AGAR WELLS**



**FIG. 25 AFTER PREPARATION
OF AGAR WELLS**

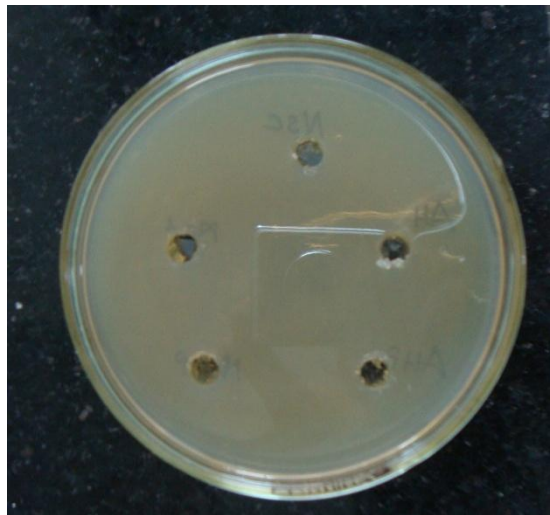


FIG. 26 SIX PETRI DISHES, ALL WITH PREPARED AGAR WELLS

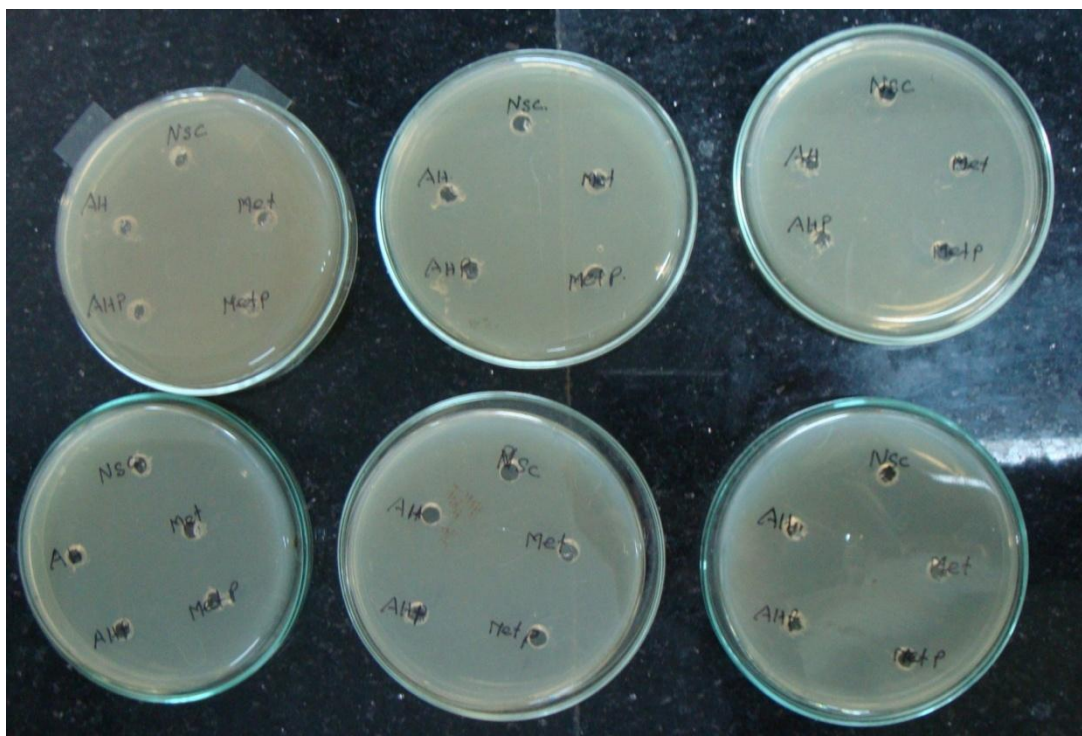


FIG. 27 MICROPIPETETTE TO DISPENSE PROPOLIS AND INOCULATE



FIG. 28 INOCULATION OF SEALERS IN AGAR WELLS



FIG. 29 INCUBATOR



FIG. 30 CULTURE PLATES IMMEDIATELY AFTER INOCULATION

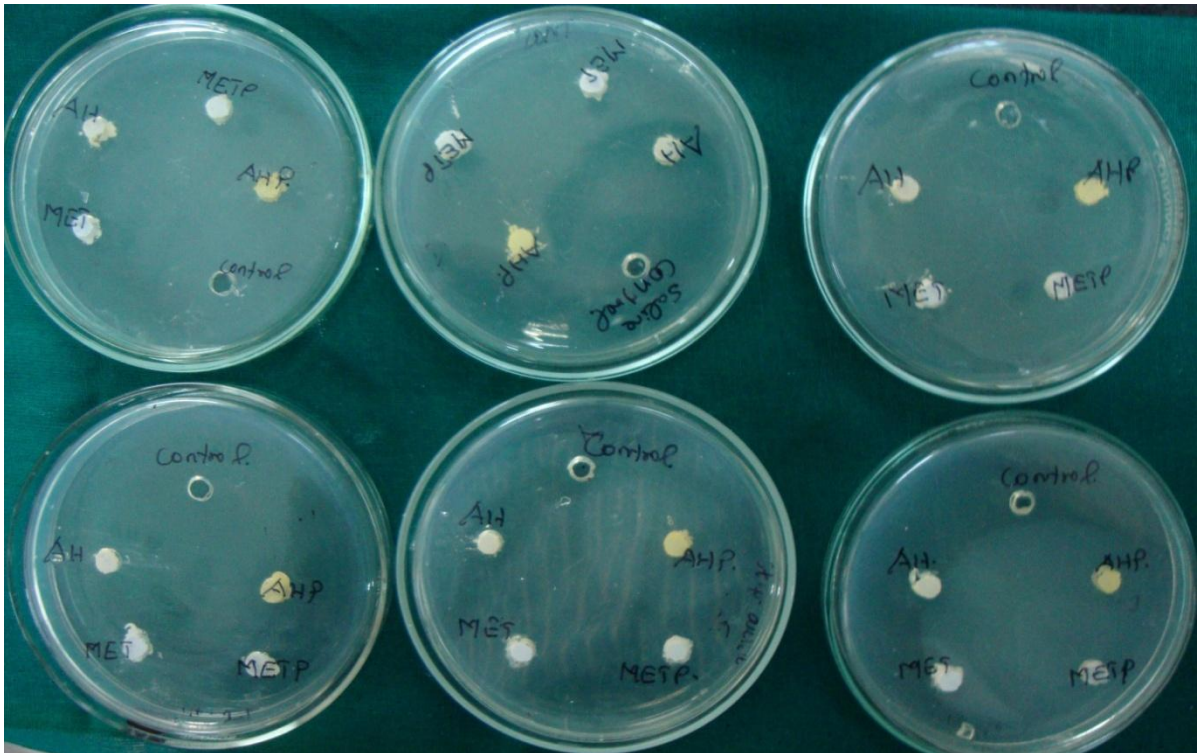


FIG. 31 CULTURE PLATES AFTER 24 HOURS OF INOCULATION

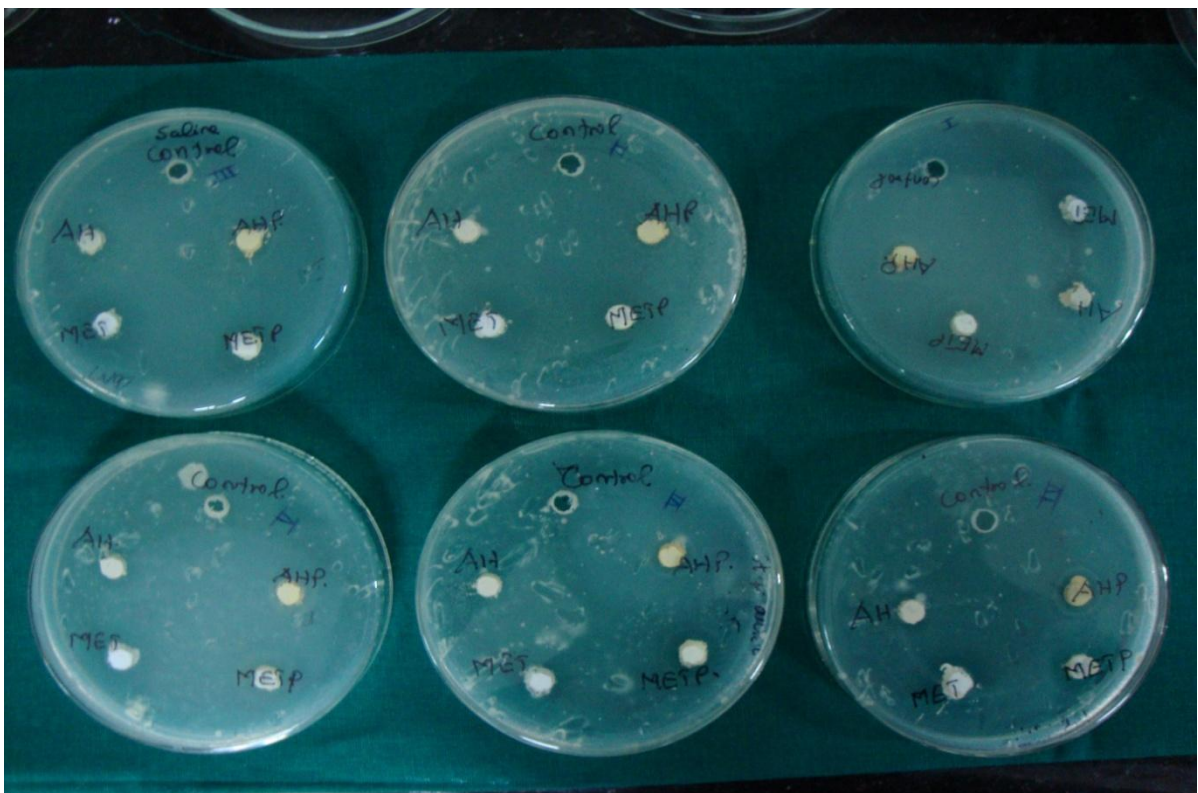


FIG. 32 CULTURE PLATE AFTER 24 HOURS SHOWING ZONES OF INHIBITION

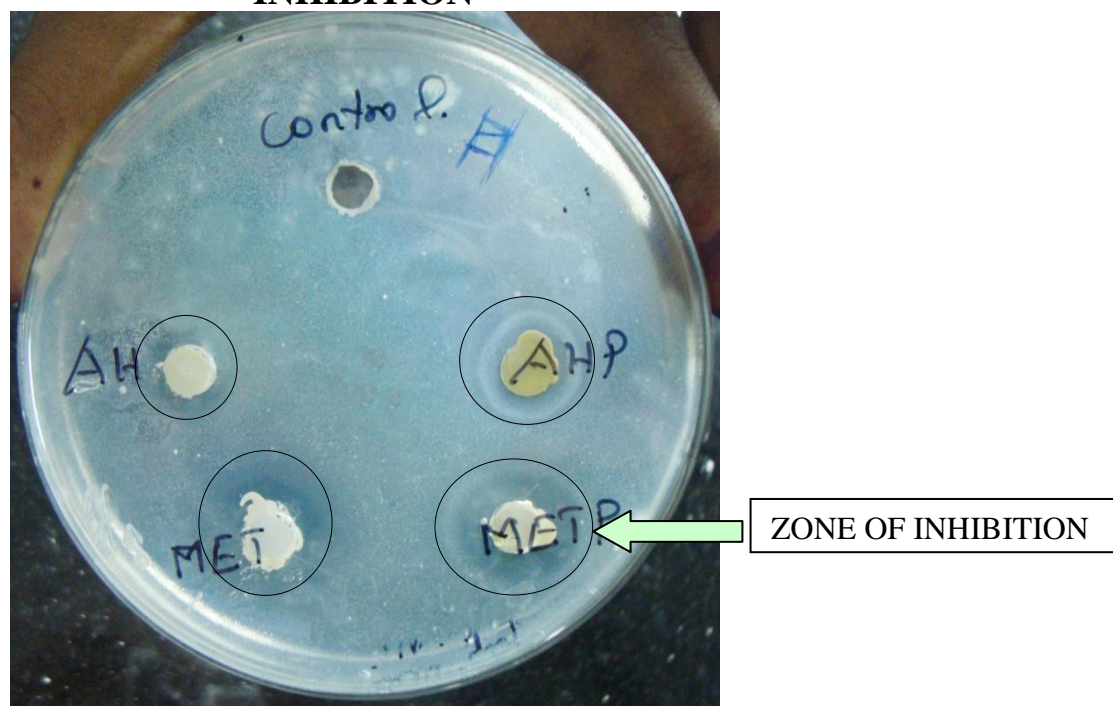
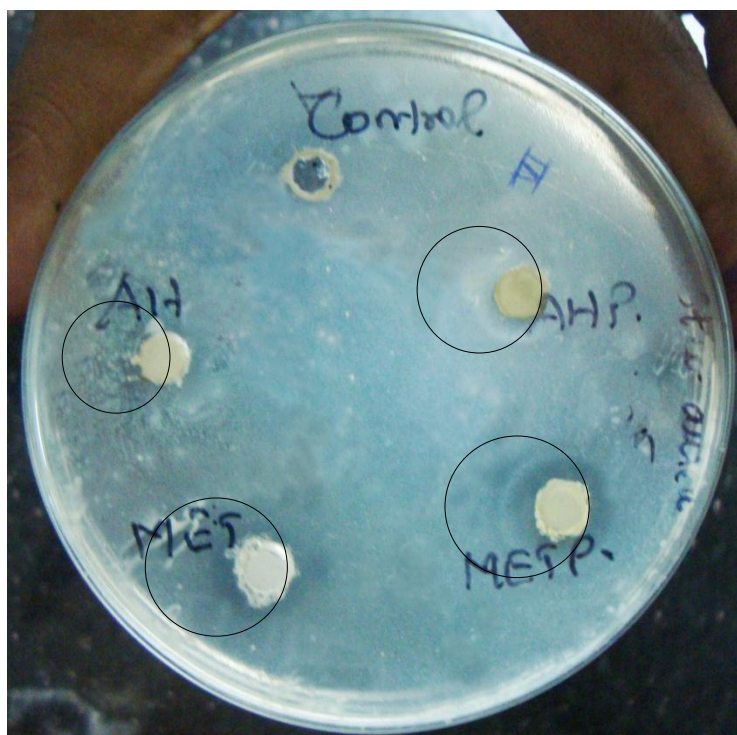
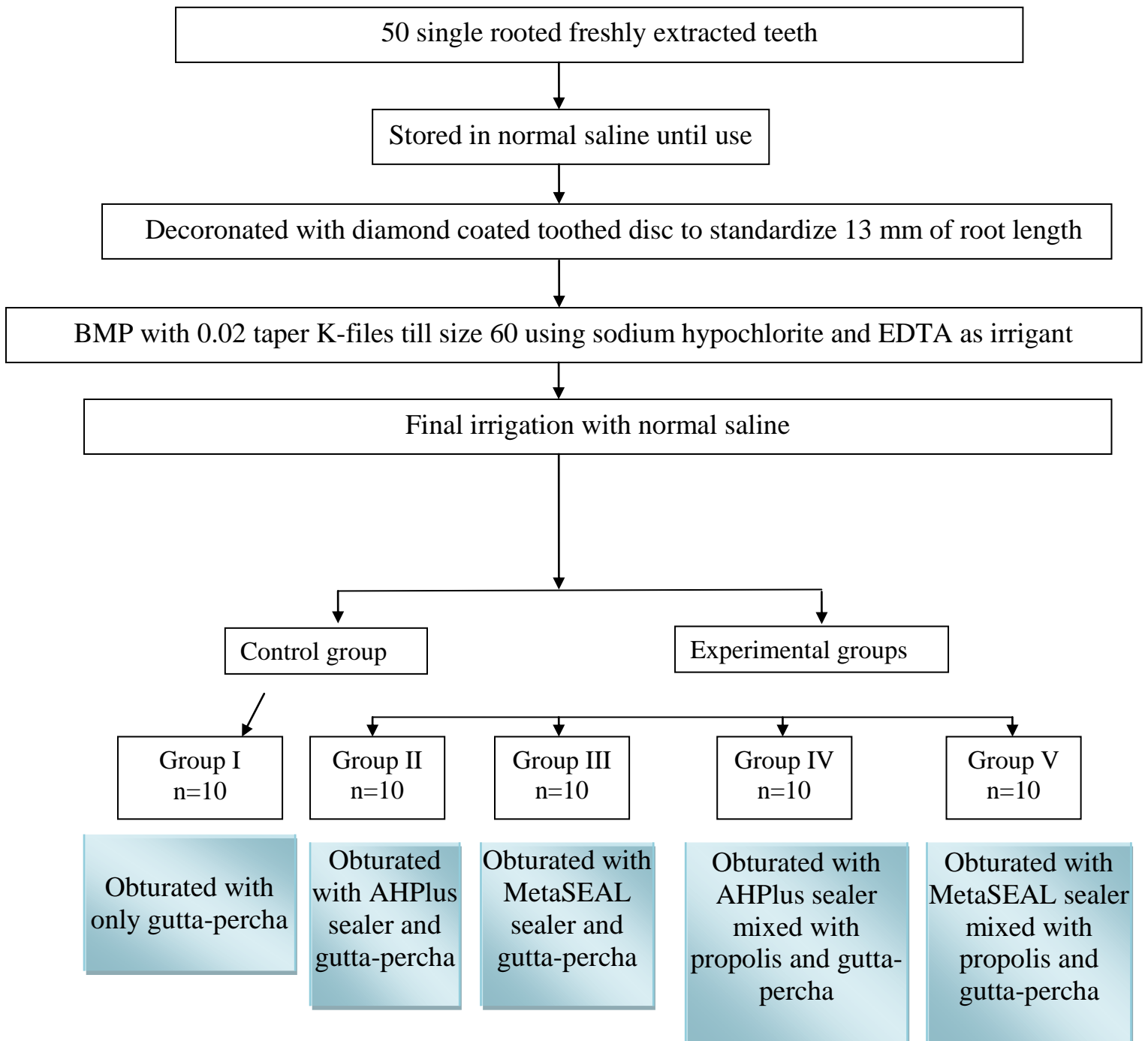


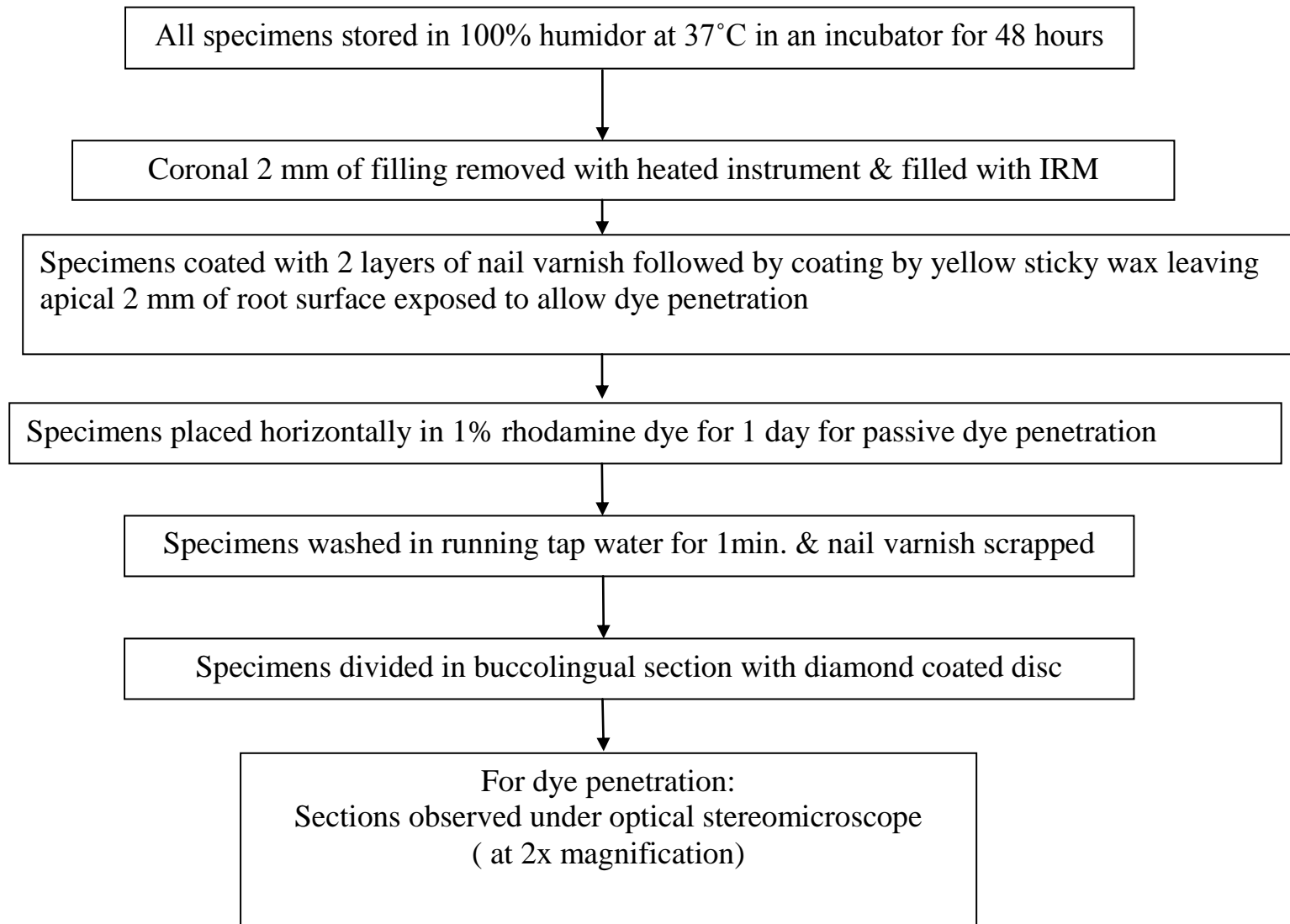
FIG. 33 CULTURE PLATE AFTER 48 HOURS SHOWING ZONES OF INHIBITION



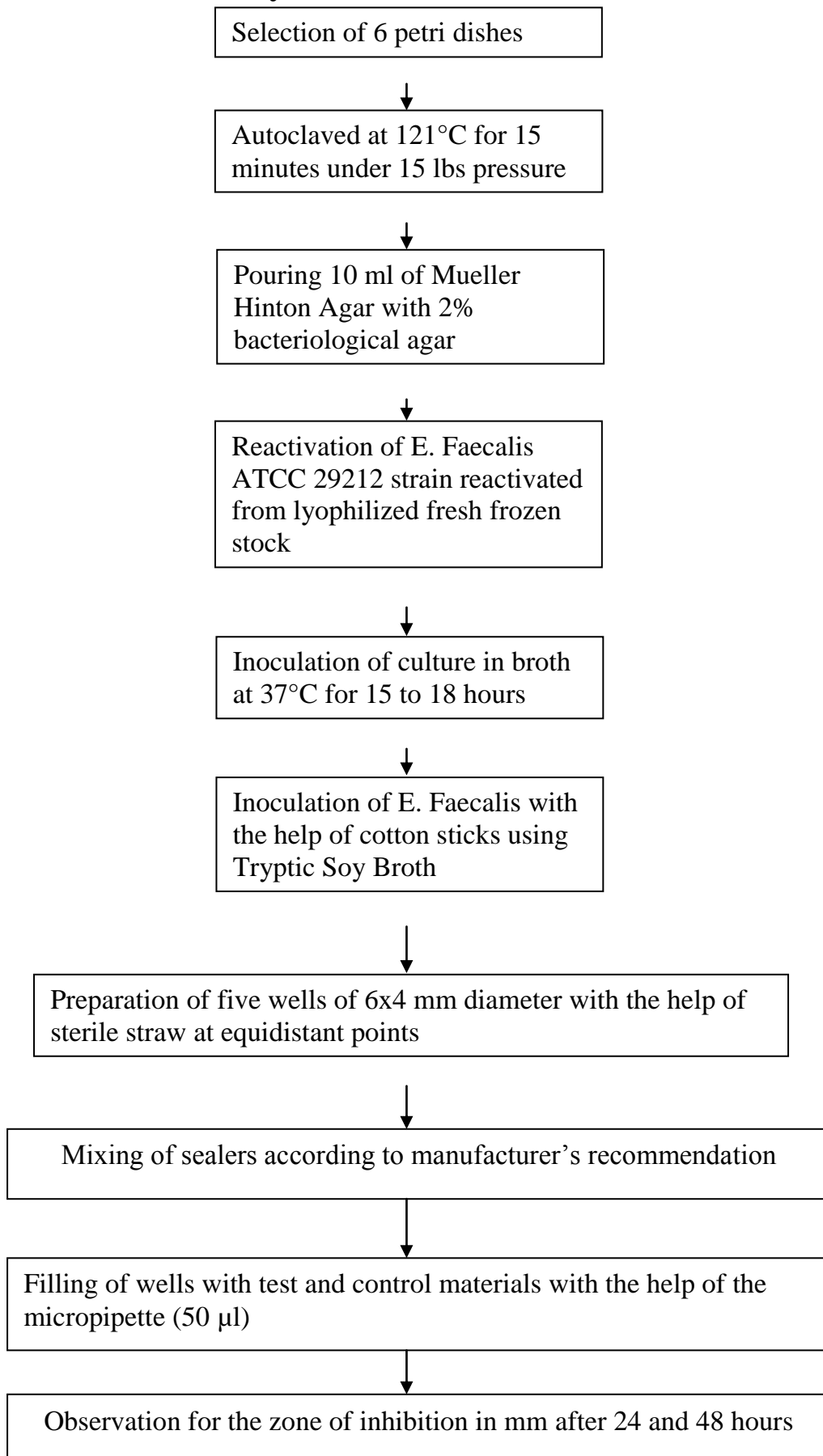
INVESTIGATION DESIGN

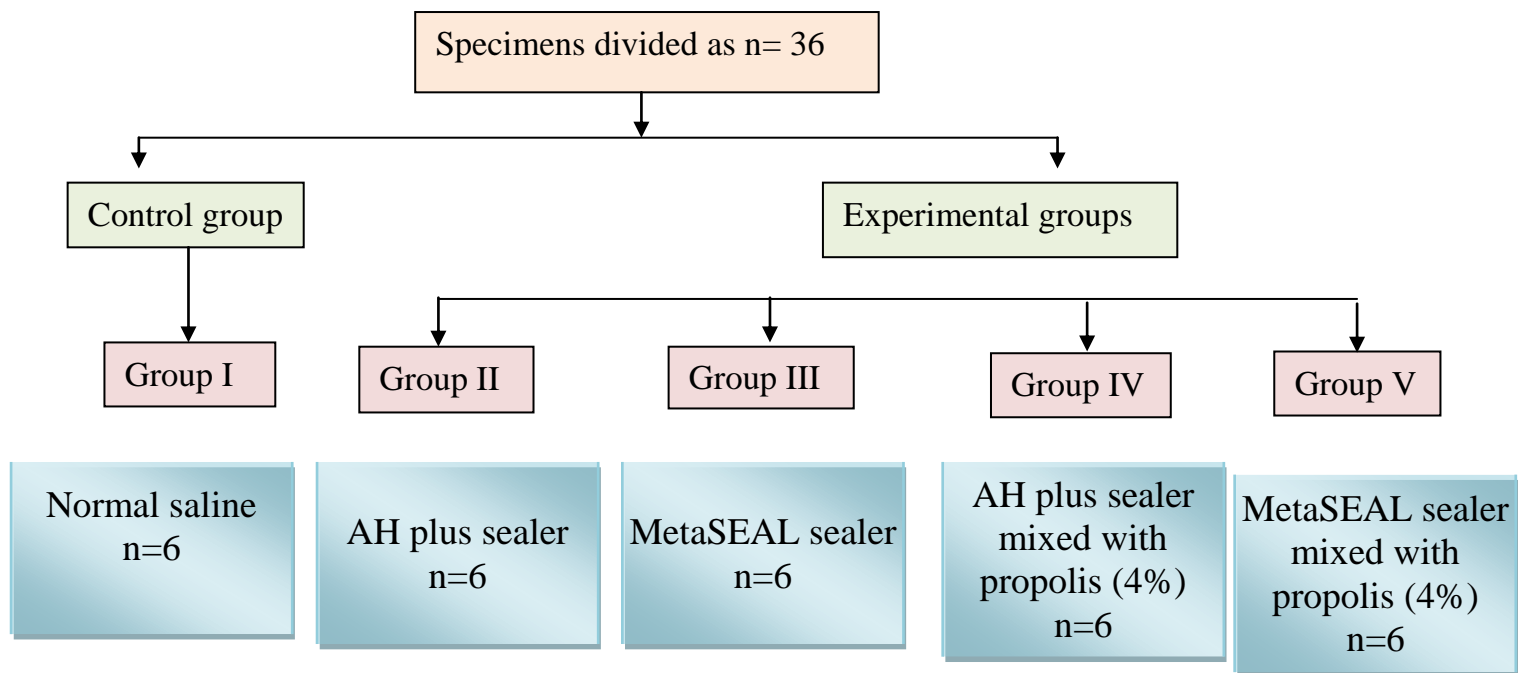
For sealing ability determination





For antibacterial activity





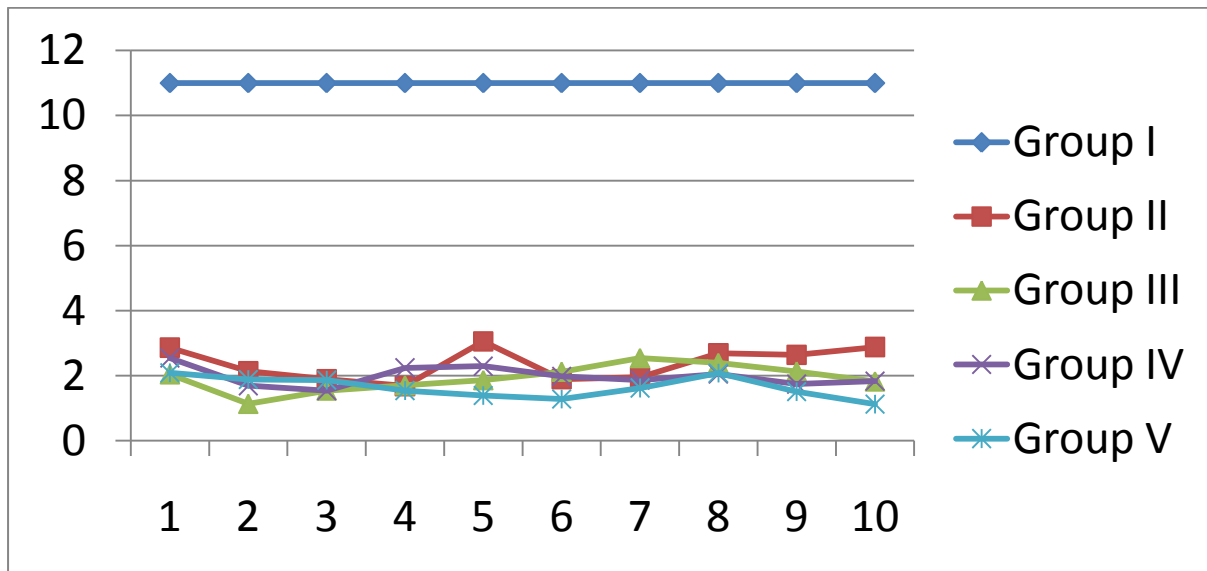
Results

FOR SEALING ABILITY

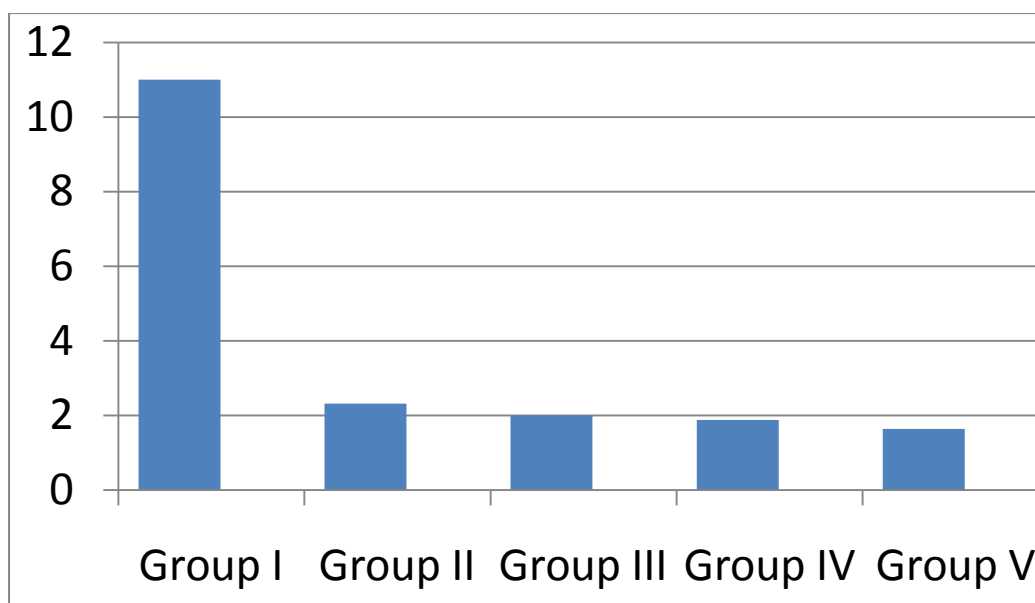
In the present study, the results of different groups observed were as follows :

TABLE I MICROLEAKAGE VALUES OF ALL SPECIMENS(mm)

	Samples	I	II	III	IV	V	VI	VII	VIII	IX	X	MEAN
Group I	Gutta-percha alone	11	11	11	11	11	11	11	11	11	11	11
Group II	AH plus	2.86	2.14	1.89	1.68	3.05	1.89	1.95	2.69	2.64	2.88	2.3157
Group III	MetaSEAL	2.04	1.13	1.54	1.7	1.86	2.12	2.54	2.39	2.13	1.82	1.9996
Group IV	AH plus with propolis	2.54	1.69	1.54	2.24	2.29	1.98	1.86	2.05	1.74	1.83	1.8807
Group V	MetaSEAL with propolis	2.09	1.88	1.86	1.54	1.39	1.28	1.62	2.08	1.51	1.12	1.6387



GRAPH I INDIVIDUAL SAMPLE VALUES OF APICAL MICROLEAKAGE(mm)

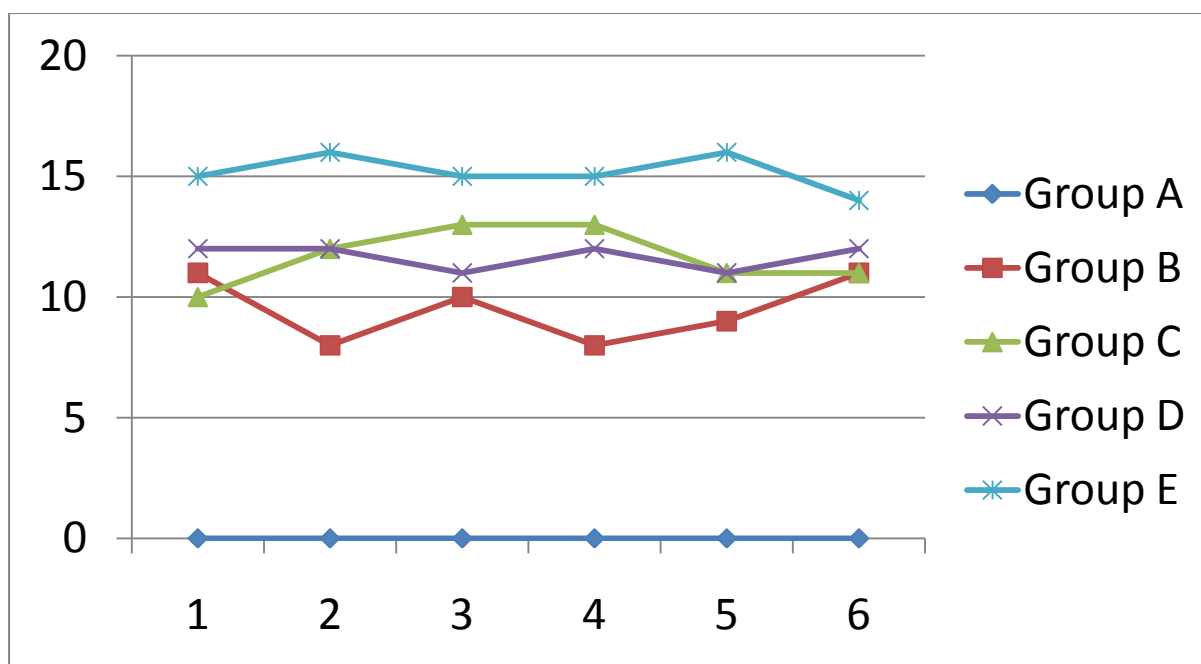


GRAPH II MEAN VALUES OF APICAL MICROLEAKAGE (mm)

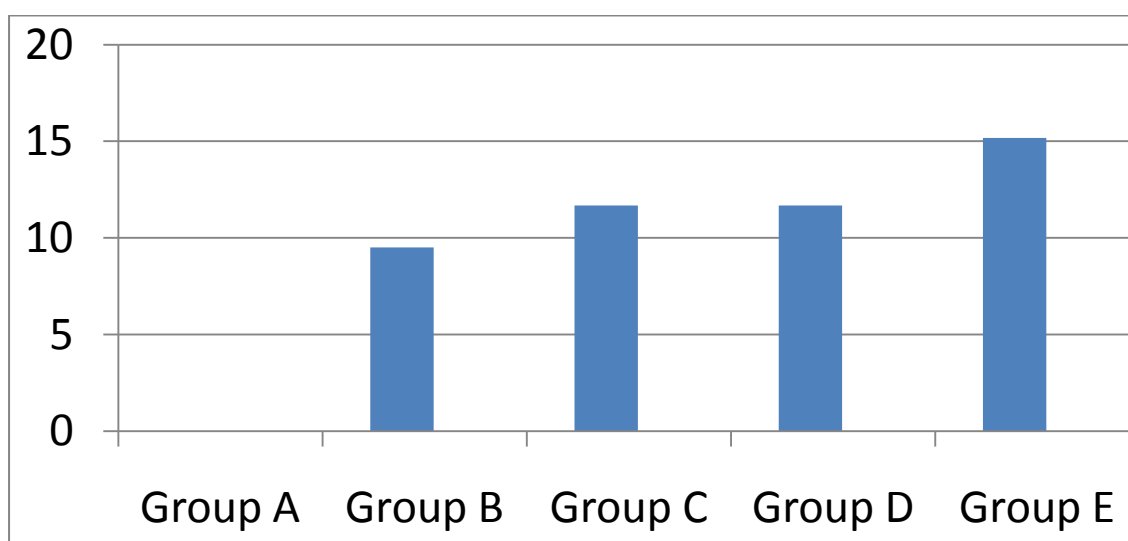
For antibacterial activity

TABLE II ZONE OF INHIBITION OF ALL THE SEALERS(mm)

		Plate I	Plate II	Plate III	Plate IV	Plate V	Plate VI	MEAN (mm)
Group A	Normal saline	0	0	0	0	0	0	0
Group B	AH plus	11	8	10	8	9	11	9.5
Group C	MetaSEAL	10	12	13	13	11	11	11.67
Group D	AH plus with propolis	12	12	11	12	11	12	11.67
Group E	MetaSEAL with propolis	15	16	15	15	16	14	15.17



GRAPH III INDIVIDUAL SAMPLE VALUES OF ZONE OF INHIBITION(mm)



GRAPH IV MEAN VALUES OF ZONE OF INHIBITION (mm)

Since, there was no difference observed between the zones of inhibition after 24 and 48 hours, the measurements taken after 24 hours were only taken into consideration for statistical evaluation.

STATISTICAL ANALYSIS

Statistical analysis of the data recorded was performed using one-way ANOVA test followed by Post-hoc multiple comparisons by Tukey HSD test. Data was analyzed using SPSS 15.0 software for statistics. Comparisons were made between all groups in a particular table for Table I and II.

FOR SEALING ABILITY:

Comparisons between different groups for apical leakage (Table I) showed no statistically significant difference in values of microleakage between Group II (AH plus sealer), Group III (AH plus sealer with propolis) and Group IV (MetaSEAL). The results were statistically significant ($p < 0.05$) when comparisons were made between Group II and Group V (MetaSEAL with propolis).

TABLE III SHOWING MEAN VALUES, STANDARD DEVIATION, STANDARD ERROR, MINIMUM AND MAXIMUM VALUES OF MICROLEAKAGE

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group II	10	2.3157	.47311	.14961	1.68	3.05
Group III	10	1.9996	.29683	.09387	1.54	2.54
Group IV	10	1.8807	.37977	.12009	1.13	2.54
Group V	10	1.6387	.33191	.10496	1.12	2.09
Total	40	1.9587	.43774	.06921	1.12	3.05

TABLE IV (ANOVA)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.376	3	.792	5.594	.003
Within Groups	5.097	36	.142		
Total	7.473	39			

TABLE V
(MULTIPLE COMPARISONS, TUKEY HSD)

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group II	Group III	.3161	.16828	.255	-.1371	.7693
	Group IV	.4350	.16828	.064	-.0182	.8882
	Group V	.6770(*)	.16828	.002	.2238	1.1302
Group III	Group II	-.3161	.16828	.255	-.7693	.1371
	Group IV	.1189	.16828	.894	-.3343	.5721
	Group V	.3609	.16828	.159	-.0923	.8141
Group IV	Group II	-.4350	.16828	.064	-.8882	.0182
	Group III	-.1189	.16828	.894	-.5721	.3343
	Group V	.2420	.16828	.485	-.2112	.6952
Group V	Group II	-.6770(*)	.16828	.002	-1.1302	-.2238
	Group III	-.3609	.16828	.159	-.8141	.0923
	Group IV	-.2420	.16828	.485	-.6952	.2112

* The mean difference is significant at the .05 level.

FOR ANTIBACTERIAL ACTIVITY:

Comparisons between different groups for antibacterial activity (Table II)

showed a statistically significant difference in values of zone of inhibition between all the groups ($p < 0.05$), except between the Group C (AH plus mixed with propolis) and Group D (MetaSEAL), which showed no significant difference.

TABLE VI SHOWING MEAN VALUES, STANDARD DEVIATION, STANDARD ERROR, MINIMUM AND MAXIMUM VALUES OF ZONE OF INHIBITION

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group B	6	9.50	1.378	.563	8	11
Group C	6	11.67	.516	.211	11	12
Group D	6	11.67	1.211	.494	10	13
Group E	6	15.17	.753	.307	14	16
Total	24	12.00	2.284	.466	8	16

TABLE VII (ANOVA)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	99.000	3	33.000	31.429	.000
Within Groups	21.000	20	1.050		
Total	120.000	23			

**TABLE VIII
(MULTIPLE COMPARISONS, TUKEY HSD)**

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group B	Group C	-2.17(*)	.592	.008	-3.82	-.51
	Group D	-2.17(*)	.592	.008	-3.82	-.51
	Group E	-5.67(*)	.592	.000	-7.32	-4.01
Group C	Group B	2.17(*)	.592	.008	.51	3.82
	Group D	.00	.592	1.000	-1.66	1.66
	Group E	-3.50(*)	.592	.000	-5.16	-1.84
Group D	Group B	2.17(*)	.592	.008	.51	3.82
	Group C	.00	.592	1.000	-1.66	1.66
	Group E	-3.50(*)	.592	.000	-5.16	-1.84
Group E	Group B	5.67(*)	.592	.000	4.01	7.32
	Group C	3.50(*)	.592	.000	1.84	5.16
	Group D	3.50(*)	.592	.000	1.84	5.16

* The mean difference is significant at the .05 level.

Discussion

DISCUSSION

Three-dimensional sealing of the root canal system is one of the main goals of endodontic treatment and is essential for prevention of canal re-infection and maintenance of healthy periapical tissues. For such purpose, several types of endodontic sealers have been developed and the evaluation of the apical sealing ability of these materials is extremely important. Therefore, leakage studies that investigate the sealing properties of endodontic materials are still considered important and relevant.⁶⁷

Apical leakage investigations of endodontic materials provide useful information about its adherence to the dentinal walls and microvoids at the interface⁵⁵. Different methods have been used to evaluate the sealing of endodontic cements. Assessment of linear dye penetration is a common method used to explore apical leakage of root fillings after splitting the roots.⁶⁷

In addition to eliminating microorganisms that have been left behind after chemomechanical preparation, a filling material should prevent recolonization of the root canal system⁷.

Microorganisms invading the root canal space may be on the dentinal walls or deep in tubules^{63,3}.

Bystrom and Sundqvist¹¹ found *E. faecalis* to be highly resistant to antimicrobial medicaments, such as calcium hydroxide. Therefore, the presence of *E. faecalis* at the time of obturation can significantly reduce the success rate of root canal treatment.

Efforts to eliminate bacteria from the root canal system are accomplished by thorough cleaning and shaping of the root canal followed by an interim dressing of calcium hydroxide and adequate filling of the empty space. The purpose of sealing root canals is to prevent periapical exudates from diffusing into the unfilled part of the canal, to prevent reentry and colonization of bacteria, and to prevent residual bacteria from reaching the periapical tissues ³².

To accomplish an airtight seal of the root canal, sealer is needed to eliminate gaps between the core filling material and the canal walls.

E. faecalis was chosen for the study because of its high resistance to a wide range of microbial agents,³⁶ its presence in association with persistent apical periodontitis,³³ its difficult elimination from the root canal with use of chemomechanical procedures,²³ and for its ease in culturing and manipulation.⁵⁹

The agar diffusion method has been widely employed to investigate the antimicrobial activity of dental materials, however; this procedure does not depend only on the material toxicity to a given microorganism, but may also be influenced by the diffusion and affinity of the material in the culture medium. A

material presenting easier diffusion will produce larger zones of inhibition of bacterial growth⁷⁰.

Epoxy resin based sealers (AH 26) were introduced because of its advantages such as high radiopacity, low solubility, slight shrinkage and antimicrobial efficacy³⁹. The antimicrobial efficacy of AH 26 is attributed to the release of formaldehyde. However, formaldehyde is a known mutagenic and carcinogenic agent⁴⁸. Hence, this sealant has been replaced by AH PLUS an improved epoxy resin sealant. AH PLUS has retained the epoxy resin "glue" of AH 26 and also is free of formaldehyde release.⁴

One of the factors that was instrumental in the development of resin-based sealers was the recognition that gutta-percha does not bond to dentin or to any conventionally used sealer, such as zinc oxide-eugenol (ZOE)-based cements and epoxy resins such as AH-26 or AH Plus. Although these materials are being used successfully, an ideal root canal sealer should be capable of bonding to root canal dentin and to gutta-percha, thus preventing microleakage. Recent advances in adhesive technology have led to the introduction of a new generation of endodontic sealers and filling materials, that are based on adhesive properties and polymer resin technology. These materials are capable of forming a hybrid layer and penetrating deep into dentinal tubules by virtue of their hydrophilic properties.¹⁵

The fourth generation methacrylate resin-based sealers are functionally analogous to a class of recently introduced self-adhesive resin luting composites in that they have further eliminated the separate etching/ bonding step⁵⁹. Acidic resin monomers that are originally present in dentin adhesive primers are now incorporated into the resin-based sealer/composite to render them self-adhesive to dentin substrates. The combination of an etchant, a primer, and a sealer into an all-in-one selfetching, self-adhesive sealer is advantageous in that it reduces the application time as well as errors that might occur during each bonding step. MetaSEAL is the first commercially available fourth generation selfadhesive dual-cured sealer^{65,53}. The inclusion of an acidic resin monomer, 4-methacryloyloxyethyl trimellitate anhydride (4-META), makes the sealer self-etching, hydrophilic, and promotes monomer diffusion into the underlying intact dentin to produce a hybrid layer after polymerization. The sealer purportedly bonds to thermoplastic rootfilling materials as well as radicular dentin via the creation of hybrid layers in both substrates. MetaSEAL is also marketed as Hybrid Bond SEAL (Sun Medical Co Ltd, Shiga, Japan) in Japan and had been reported to produce similar or slightly inferior sealing properties as conventional nonbonding epoxy resin-based sealers^{50,8}.

This study used the conventional method of dye penetration for evaluating apical leakage observed under stereomicroscope and agar diffusion test for

evaluating antibacterial activity against *E. Faecalis*, of two resin based sealers, namely AH plus and MetaSEAL mixed with propolis.

Fluid filtration model despite its limitation in not providing information about the interface location of the leakage, it is still used by the some of the investigators.^{18,44,64}

Rhodamine B dye was used as a leakage marker in the present study because it presents greater diffusion on human dentin than methylene blue. According to Francci,²⁵ its molecules are nanometric and are optimal to simulate enzymes and toxins of leakage resulting from bacterial metabolism. According to Azoubel and Veeck,⁶ rhodamine B dye should be used in leakage studies because of its small particle size, ease of visualization, and large dissemination into dentinal tubules. It has low molecular weight particles, which could represent the spread of toxic by-products into micro spaces between the root filling and root canal walls.

The smear layer resulting from root canal instrumentation acts as a physical barrier interfering with the adaptation and penetration of the sealer into the dentinal tubules, which might contribute for increasing microleakage occurrence. Use of chemically active, adhesive root canal sealers may play an important role in minimizing apical leakage. In this study, the smear layer was

removed from the specimens with 17% EDTA. By doing so, the surface contact between the intracanal walls and the filling material is increased and apical seal may be improved²⁴.

Emel olga onay et al. also showed that there is significantly less microleakage when EDTA was used as a final irrigant compared to Er,Cr:YSGG laser irradiation.²²

Propolis, used in this study has already been used in dentistry for the repair of surgical wounds, for treatment of infected root canal and apical periodontitis, for dental socket wounds, for direct and indirect pulp capping and for dental hypersensitivity.⁴⁵

Several investigators have also used it as a storage medium of avulsed teeth³⁰, as an intracanal medicament⁴² or irrigant³⁷.

Propolis is derived from the Greek word “pro” before, polis “city” or defender of the city. Propolis is the glue that honey bees (*Apis mellifera*) use to seal up their hives.

It is composed of resin (55%), essential oils and wax (30%) mixed with bee glue “the salivary secretions of bees” and pollen (5%) and other constituents (10%) which are amino acids, minerals, ethanol (alcohol), vitamins A, B

complex, E and the highly active bio-chemical substance known as bioflavenoid.⁷⁵

The results of this study stated that all the sealers produced microleakage and all of them showed wide variety of leakage values. The mean difference was remarkable in leakage with least of 1.6387 mm was seen in specimens obturated by MetaSEAL and propolis, whereas greatest mean leakage was seen in AH plus group, about 2.3157 mm.

However, addition of propolis to the sealers did not show any significant effect on sealing ability of particular sealer or between the sealers themselves. But, there was significant difference between AH plus and MetaSEAL mixed with propolis.

The sealer AH Plus is based on epoxy amine resins and has been used with gutta-percha points for root canal obturation for many years. Miletic et al. reported that AH plus exhibited greater, but not statistically significant, leakage than samples filled with AH 26⁵⁷. Similar results have been reported by Zmener et al.⁶¹. This was explained by the faster setting time of AH Plus, which caused shrinkage stress and earlier debonding from dentine walls.. Miletic et al. showed that after 1 year, AH plus indicated significantly better sealing ability than

Apexit, whereas AH 26 and Diaket had no statistical differences with either sealer⁵⁵. They stated that AH plus showed satisfactory sealing ability.

Sema belli et al. compared the long term sealing ability of AH plus with MetaSEAL using fluid transport model and they found no significant difference in microleakage between these sealers after 24 weeks.

Our study is in accordance to the previous study where there is no significant difference found between the apical leakage of these two sealers.

Still there was some amount of leakage observed in all the specimens. Reason may be given as the chemical coupling between contemporary methacrylate resin-based sealers to root filling materials is generally weak or insufficiently optimized. In view of the extremely high C-factor encountered in long, narrow root canals, it is doubtful whether the core material–sealer bond is capable of resisting polymerization shrinkage stresses that develop during the setting of the resin sealer to permit the realization of the goal of creating a monoblock in the root canal system.

Richard Stoll et al. found that there is no significant difference between MetaSEAL bond to either resilon or gutta-percha. In this study gutta-percha was used in conjunction with MetaSEAL.⁶⁶

Also Hale Ari et al. noted that apical sealing of MetaSEAL is superior with cold lateral condensation and vertical compaction when compared with Thermafil and Ultrafil techniques. In this study cold lateral compaction technique was used for obturation.³⁴

When comparing the antibacterial activity a study by Stuart⁷ showed that the resin based sealers AH Plus and RC seal showed no zones of inhibition against both *Enterococcus faecalis* and *Candida albicans*. The elimination of formaldehyde release from AH plus has made it an ineffective antimicrobial sealant. This result was in concurrence with Andre Mickel et al⁴ who found AH PLUS to be ineffective against *Enterococcus faecalis* and Kaplan et al who found AH PLUS to be ineffective against *Candida albicans*.

There are numerous studies done regarding antibacterial activity of AH plus and most of them stated that it has either very low or no antibacterial activity against *E. Faecalis*.

One study reported by Funda Kont C et al. compared the various sealers like Sultan, Roekoseal, Sealapex, AH plus and Ketac endo. They found out that AH plus has same antibacterial effect as other sealers and it showed complete inhibition of growth for at least first 19 hours.²⁶

Till this time, there is no evidence of antibacterial activity of MetaSEAL evaluated by any investigator in literature. However, in this study there was significant amount of difference observed between the antibacterial activity of AH plus and MetaSEAL. This finding may be attributed to 4- META content of the sealer which itself may be cytotoxic to the tissues and leaching out from the material in small amounts until set.

However, this cytotoxicity reduces over time and as material sets in the root canal.²

However, still adding propolis to the sealer has identified its improved activity against *E. Faecalis*, which has been supported by various investigations done on propolis.

Lama Awawdeh et al. used propolis as an intracanal medicament and they compared the antibacterial activity of propolis with calcium hydroxide as a short-term intracanal medicament and they concluded that natural bee product propolis is very effective *ex vivo* in elimination of *E. Faecalis* in 24 hours compared to calcium hydroxide.⁴⁷

After addition of propolis to sealers, their increased antibacterial properties can be explained by the properties of propolis itself.

Propolis extracts have been reported to potentiate some antibiotic effects attributing the antibacterial propolis activity mainly to flavonoids or to a synergism between some components.⁴¹

Another compound in propolis **totarol** is a known antimicrobial agent against Gram positive bacteria, and totarol isolated from Greek propolis showed a specific activity against *S. aureus* and *S. epidermidis*, comparable to that of standard antibiotics. This also may be the reason of high antibacterial activity of propolis containing sealer groups.¹⁶

Hence, within the limitation of this study it can be said that MetaSEAL was not superior to AHPlus as far as the sealing ability is concerned and adding propolis to those sealers did not have any significant effect on their sealing ability. However, AH plus has shown inferior antibacterial activity compared to MetaSEAL and addition of propolis to these sealers have significantly enhanced their antibacterial activity against *E. Faecalis*.

Summary

This study was undertaken to compare the sealing ability and antimicrobial activity against *E. Faecalis* of two resin based sealers, namely AH plus and MetaSEAL mixed with an antibacterial natural product, propolis.

For evaluating apical sealing ability 50 freshly extracted single rooted human anterior teeth were used. The anatomical crowns were resected at cemento-enamel junction and divided into 4 experimental groups and one control group, each containing 10 samples.

Out of these in first group no sealer was used and canals were obturated using only gutta-percha cones. In next two groups AH plus and MetaSEAL sealers were used respectively. In last two groups AH plus and MetaSEAL sealer mixed with propolis were used and obturated using gutta-percha cones. Dye penetration was performed using 1% rhodamine dye. The roots were sectioned longitudinally and examined under 2x magnification by stereomicroscope and dye penetration was recorded in mm and results tabulated. Statistical analysis was performed using one-way ANOVA test followed by Post-hoc multiple comparisons by Tukey HSD test

For evaluating antimicrobial activity of sealers against *E. Faecalis*, they were again divided into five groups. First group contained only normal saline as a control. Second and third contained AH plus and MetaSEAL respectively, whereas fourth and fifth group was these sealers mixed with propolis.

E. Faecalis ATCC 29212 strain was reactivated and cultured for 24 hours from fresh frozen stock. This strain was inoculated using trypticase soy broth. After preparation of five agar wells in 6 plates, sealers were mixed and four wells were inoculated using experimental sealers and fifth with normal saline as control with the help of a micropipette. After inoculation zone of inhibition was observed in mm at 24 and 48 hours intervals with a ruler.

Conclusion

Within the limitations of this study the following conclusions could be drawn:

- No sealer provided complete apical seal and both sealers AH plus and MetaSEAL showed apical microleakage.
- There was no significant difference between the apical microleakage observed between AH plus and MetaSEAL.
- Mixing of propolis with both the sealers does not significantly affect the apical sealing ability of that particular sealer.
- MetaSEAL mixed with propolis showed significantly lesser apical leakage than AH plus alone.
- MetaSEAL showed significantly greater antimicrobial activity against *E. Faecalis* than AH plus.
- Adding propolis to the sealers, AH plus and MetaSEAL significantly enhanced their antimicrobial activity against *E. Faecalis*.

However, in this in vitro study only two parameters of sealers were evaluated after mixing with propolis. Further studies are required to evaluate its properties such as film thickness, bond strength, radiopacity, solubility etc.

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